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Intracavernosal mesenchymal stem cell therapy in ischaemic priapism: an experimental study

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Abstract

Introduction The most common form of priapism is ischaemic and its prevalence in men has increased in recent years as a result of intracavernosal drug use. Currently, there is no approved specific treatment for ischaemic priapism other than cavernosal aspiration, which can only provide detumescence. This study aims to evaluate the efficacy of intracavernosal mesenchymal stem cell (MSC) therapy in an ischaemic priapism model.

Material and methods Thirty male Wistar albino rats were divided into three groups: sham ($n=6$), priapism ($n=12$) and priapism + MSC treatment ($n=12$). The experimental groups were also divided into 1 and 12 h subgroups of ischaemic priapism. The experimental model was created using a vacuum erection device and constrictive tape technique, and intracavernosal MSC were applied immediately after the tape was removed. After 4 weeks, intracavernosal pressures (ICPs) and systemic mean arterial pressure (MAP) were measured. Penectomy was then performed to assess histopathological and molecular changes in the rats' penile tissues.

Results In the ischaemic priapism model, MSC therapy showed significant improvements in peak and mean ICPs and mean ICP/MAP ratio. Histopathological analysis showed significant increases in smooth-muscle/collagen ratio and e-NOS and n-NOS expression. Although there was a decrease in fibrosis, it was not significant. At the molecular level, there were significant decreases in TGF-beta and VEGF mRNA expression, whilst NGF and BDNF mRNA-expression levels showed significant increases with MSC therapy. In terms of ICPs, the therapy showed more significant improvements in short-term priapism. However, when looking at histopathological and molecular parameters, the therapy had positive effects on a wider range of parameters in the long-term priapism.

Conclusion MSC treatment improved cavernosal physiology and had positive effects at the histopathological and molecular level in the ischaemic priapism model.

Keywords Priapism · Mesenchymal stem cell · Treatment · Penile erection

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Introduction

Priapism is a condition in which a penile erection lasts for more than 4 h, regardless of sexual desire or arousal [1]. It can occur at any age but tends to affect younger men more frequently than those over 40 and has three types: ischaemic priapism, non-ischaemic priapism and recurrent priapism [2]. Ischaemic priapism, the most common type, is characterised by low blood flow and pain and requires immediate medical attention [3]. Untreated or inadequately treated ischaemic priapism can lead to erectile dysfunction due to tissue damage and fibrosis [4, 5]. The major complication of this condition is penile fibrosis, which will eventually lead to erectile dysfunction. Although this is the most dangerous outcome, effective strategies to prevent fibrosis in cases of ischaemic priapism are limited due to the complex molecular mechanisms of penile fibrosis [5]. Several medical therapeutic approaches, including phosphodiesterase-5 inhibitors (PDE5I), pentoxifylline, a non-specific cyclic AMP (c-AMP) phosphodiesterase (PDE) inhibitor, and vasoactive modalities such as prostaglandin E1 (PGE1), have been proposed for the treatment of cavernosal fibrosis secondary to various aetiologies. Although they have a favourable effect on the progression of cavernosal fibrosis in the early stages, most cases eventually require corporal reconstruction surgery with concomitant penile prosthesis placement [6, 7].

In this regard, more effective medical approaches are still needed for the treatment of ischaemic priapism and associated cavernosal fibrosis. Several preclinical studies have shown promising results using stem-cell therapy to improve functional and histological outcomes in fibrosis and erectile dysfunction associated with conditions such as diabetes, ageing, cavernosal nerve damage and Peyronie's disease [8–11]. However, there is limited research on stem-cell therapy specifically for priapism [12]. The aim of this study is to investigate the effect of intracavernosal mesenchymal stem cell (MSC) therapy on functional and histological recovery in an ischaemic priapism model.

Material and methods

Study design and establishment of the priapism model

In this study, 30 adult Wistar albino rats, weighing 250–300 g and age 6–10 weeks were used. The rats were maintained on standard rat chow and had ad libitum access to water. They were housed in standard rat cages at a temperature of 20 ± 2 °C with a 12-h light/dark cycle. To establish the ischaemic priapism model, we followed the methodology previously described by Sanlı et al. under anaesthesia [13]. For anaesthesia, the rats were given xylazine hydrochloride (Rompun 2%, Bayer HealthCare Pharmaceuticals Inc., Istanbul, TR) at a dose of 10 mg/kg and ketamine hydrochloride (Alfamine 10%, Ege Vet Ltd., Istanbul, TR) at a dose of 50 mg/kg for sedation and smooth-muscle relaxation and dissociative anaesthesia. Rats were randomly divided into three groups: Group 1 (sham group), Group 2 (priapism control group) and Group 3 (priapism treatment group). Compared to humans, rodents have a relatively short and accelerated lifespan. Rats typically live up to 3 years (1095 days), whilst humans live about 80 years (29,200 days). Rats live about 27 times faster, meaning that 1 rat day is equivalent to about 27 human days [14]. In line with this time frame, a priapism model was created in this study to represent the early stage, which lasts 1 h, and the late stage, which lasts 12 h.

Group 2 and 3 were further divided into short-term and long-term priapism groups. The sham group received intracavernosal 0.2 cc saline only, whilst the priapism control group underwent the priapism model procedure followed by intracavernosal 0.2-cc saline injection. The priapism treatment group underwent the priapism model procedure followed by intracavernosal MSC therapy (0.2 cc mesenchymal stem-cell solution). The amount and route of mesenchymal stem cell (MSC) administration was determined based on previous experimental studies of penile fibrosis [15]. After 4 weeks, all rats underwent cavernosal electrophysiological pressure measurement followed by penectomy. Histopathological examination and polymerase chain reaction (PCR) analysis of tissue mRNA expression were also performed.

Isolation of mesenchymal stem cells

For the isolation of MSCs, the femurs of rats from the control group were removed at the end of the experiments and sacrificed. The bone marrow was collected in a culture dish with medium and incubated overnight. The next day, non-adherent cells were discarded and the remaining cells were washed with phosphate-buffered saline (PBS). The adherent cells were separated and characterised by flow cytometry analysis, which characterised the surface markers of mesenchymal stem cells (CD29, CD44, CD73, CD90 and CD105) and haematopoietic surface markers (CD11b, CD31, CD34, CD45 and CD103). The multipotent properties of the cells were demonstrated by their ability to differentiate into endothelium, adipose tissue and bone tissue.

Electrophysiological pressure measurement

Electrophysiological pressure measurements were also performed under the same anaesthesia as described above. To describe mean arterial blood pressure (MAP), the left carotid artery was cannulated with a PE50 tube connected to a pressure transducer and amplifier unit (COMBAT Pharmacology & Physiology Instruments, Ankara, TR). The unit was also connected to the information conversion module, MP 35 data-acquisition system (Biopac Systems, Inc., CA, USA) for pressure measurement. Intracavernosal pressures (ICPs) were measured after cavernosal nerve stimulation using a 25-gauge needle inserted into the corpus cavernosum and connected to the manometer. After accessing the greater pelvic ganglion, the cavernosal nerve was isolated and placed on a bipolar electrode. The electrode cable was connected to the STPT02 stimulator (COMBAT Pharmacology & Physiology Instruments, Ankara, TR) and the stimulation parameters were 1.5 milliampere, 15 Hz, 5 ms pulse interval, 35 ms delay, 2.5 V for 60 s. MAP and ICPs including basal intracavernosal pressure (ICP basal), mean intracavernosal pressure during the plateau (ICP mean) and maximum intracavernosal pressure (ICP max) were recorded using the Biopac Students Lab program (Biopac Systems, Inc., CA, USA). As the changes in systemic pressure after cavernosal nerve stimulation directly affect cavernosal pressure, the evaluation of intracavernosal pressure was also performed using the ICP-mean/MAP ratio [16].

Pathological and ımmunohistochemical evaluation

At the end of the experiments, the penises of all rats were removed and divided into two equal parts. One part was placed in 10% formaldehyde for histological examination, whilst the other part was stored in an Eppendorf tube at −80 °C for PCR analysis. Rat specimens were fixed in 10% formaldehyde solution. After the blocking and paraffin embedding procedures were applied to each sample, 4- µm thick sections were cut. These sections were stained with hematoxylin and eosin for routine examination to determine tissue inflammation and fibrosis. Inflammation was not scored as it was not observed in any of the rats. Fibrosis was scored as follows: none = 0, mild fibrosis = 1, moderate fibrosis = 2, extensive fibrosis = 3. We also calculated the ratio of smooth-muscle tissue to collagen tissue using Masson's trichrome staining. The ratio of smooth-muscle tissue to collagen tissue was calculated in a randomly selected × 20 high-power field in the cavernosal tissue. Quantitative analysis of smooth muscle and collagen areas was performed using Adobe Photoshop CS, version 8 (Adobe Inc., CA, USA). Transforming Growth Factor beta (TGF-beta), elastin, endothelial nitric oxide synthase (e-NOS), neuronal nitric oxide synthase (n-NOS) and inducible nitric oxide synthase (i-NOS) were used as immunohistochemical markers. Their expression intensity and prevalence were determined semiquantitatively using specific antibody kits on selected paraffin sections using an automated immunohistochemical staining device (BenchMark ULTRA/Ventana Medical System, Tuscon, AZ, USA). Scoring criteria according to prevalence were as follows: none = 0, 0–25% = 1, between 25 and 75% = 2, between 75 and 100% = 3 and scoring criteria according to intensity were as follows: none = 0, weak staining = 1, moderate staining = 2, strong staining = 3.

RT-PCR evaluation

After homogenisation of rat-penile tissues using the Tissue lyser LT system (Qiagen Inc, CA, USA), RNA isolation was performed using TRIzol reagent and Purelink RNA mini kit (Thermo Fisher Scientific, MA, USA). The purity of the collected RNAs was then determined using an Optizen NanoQ microvolume spectrophotometer (K Lab Keen Innovative Solutions, K Lab Co. Ltd., Daejeon, KR), and complementary DNA (cDNA) was produced using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA). The obtained cDNAs were stored for quantitative real-time PCR (qRT-PCR) study and determination of gene expressions. qRT-PCR was performed according to the protocol of Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific., MA, USA) to analyse the expression levels of TGF-beta, Vascular Endothelial Growth Factor (VEGF), Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF) genes in the control and treatment groups. The obtained gene expressions were calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH and β-actin as calibration and correction factors.

Statistical analysis

The sample size of the experimental animals used in our study was calculated as a minimum of six rats in each group. The statistical programme G Power version 3.0.10 (Department of Cognitive and Industrial Psychology, Heinrich Heine University, Düsseldorf, DE) was used to calculate the sample size. The alpha value and power were set at 0.05 and 0.8, respectively, for the analysis. SPSS statistical package program version 26 (IBM SPSS Inc., IL, USA) was used for data evaluation and statistical analysis. Descriptive statistical methods, such as mean and standard deviation (sd), median and IQR (interquartile range), and percentiles, were used in the presentation of the data. The distribution of the data was assessed using the Shapiro–Wilk test and all numerical parameters showed a normal distribution. An independent *t* test was used for these numerical parameters, which showed a normal distribution in paired group comparisons. The Mann–Whitney *U* test was used to compare ordinal score

data obtained after histopathological and immunohistochemical examination between paired groups. The chi-squared test was used to compare categorical data. Statistical significance was accepted as 95% confidence interval ($p < 0.05$).

Results

A total of 30 rats were included in the study, divided into three main groups: sham ($n = 6$), priapism ($n = 12$), and priapism + treatment group ($n = 12$). Within the priapism and priapism + treatment groups, separate subgroups were created with 1- and 12-h priapism models ($n = 6$, for each). All rats successfully completed the experimental protocols and were sacrificed at the end of the study.

Comparative analysis of the sham and priapism models

We first compared the sham group with the priapism group to evaluate the effectiveness of our experimental priapism model. The MAP of the rats showed no significant difference. However, statistically significant decreases in ICPs and ICP mean/MAP ratio were observed in rats with the priapism model (ICP basal = 24.8 ± 3.7 mmHg vs.15 0.8 ± 2.6 mmHg, $p < 0.001$; ICP mean = 56.2 ± 5.5 mmHg vs. 26.2 ± 5 mmHg, $p < 0.001$; ICP max = 94.9 ± 22.8 mmHg vs. 31.4 ± 6.3 mmHg, $p < 0.001$ and ICP mean/MAP ratio = 0.6 ± 0.07 mmHg vs. 0.26 ± 0.06 mmHg, $p < 0.001$ for the sham and priapism groups, respectively). Histopathological findings revealed a significant increase in fibrosis score (0 ± 0 vs. 1 ± 1, $p = 0.02$) and TGF-beta expression score (0 ± 0 vs. 1 ± 2, $p = 0.04$) in the priapism group. There was also a significant decrease in elastin expression (2 ± 0 vs. 1.5 ± 1, $p = 0.04$) and e-NOS expression (2 ± 1 vs. 1 ± 0, $p = 0.01$) in rats with priapism. However, there was no difference in n-NOS and i-NOS expression between the groups. Molecular analysis showed a significant increase in TGF-beta and VEGF mRNA expressions in the priapism model compared to the sham group (4.85 ± 3.02 vs. 1.04 ± 0.3, $p = 0.008$ and 3.48 ± 0.88 vs. 1.02 ± 0.24, $p < 0.001$), whilst NGF and BDNF mRNA expressions were significantly decreased (0.61 ± 0.19 vs. 1.0 ± 0.1, $p < 0.001$ and 0.46 ± 0.11 vs. 1.01 ± 0.19, $p < 0.001$).

Short and long-term effects of the priapism model

When analysing the short-term and long-term effects of the priapism model, we observed that rats subjected to the 12-h priapism model exhibited significantly lower ICP mean and ICP max values compared to the 1-h model (22.1 ± 2.9 mmHg vs. 30.3 ± 2.5 mmHg, $p < 0.001$ and 26.8 ± 5.9 mmHg vs. 36.0 ± 1.7 mmHg, $p = 0.005$, respectively). The ICP mean/MAP ratio also significantly decreased (0.2 ± 0.03 mmHg vs. 0.31 ± 0.01, $p < 0.001$), although basal ICP and MAP showed no significant changes. Histopathological analysis indicated a significant increase in fibrosis score (1 ± 0 vs. 0 ± 0, $p = 0.006$) and a decrease in elastin expression score (1 ± 1 vs. 2 ± 0, $p = 0.02$) in the 12-h model compared to the 1-h model. The 12-h priapism model also showed decreases in the smooth muscle/collagen ratio, e-NOS and n-NOS expression scores, and an increase in TGF-beta expression, though these changes were not statistically significant. At the molecular level, there was a significant increase in TGF-beta and VEGF mRNA expression in the 12-h priapism model (7.61 ± 1.2 vs. 2.09 ± 0.53, $p < 0.001$ and 4.03 ± 0.94 vs. 2.93 ± 0.34, $p = 0.02$, respectively), whereas NGF and BDNF mRNA expression showed no difference. According to our findings, long-term ischaemic period was more harmful in the priapism model.

Effectiveness of MSC therapy in the priapism model

MSC therapy showed significant improvements in maximum and mean cavernosal pressures and mean-ICP/MAP ratio, which are electrophysiological parameters used to assess the efficacy of therapy in the priapism model. There were no significant changes in basal ICP and MAP (Fig. 1). Histopathological analysis showed a significant increase in smooth-muscle/collagen ratio and e-NOS and n-NOS expression scores with MSC treatment (Table 1). Although fibrosis scores decreased with treatment, this reduction was not statistically significant. Molecular analysis revealed a significant decrease in TGF-beta and VEGF mRNA expression, whilst NGF and BDNF mRNA-expression levels significantly increased after MSC therapy (Table 2).

Effects of mesenchymal stem-cell therapy in the short-term and long-term ischaemic priapism

It was observed that MSC therapy was more effective in short-term priapism when considering the duration of priapism. In terms of electrophysiological parameters, the therapy showed more significant improvements in short-term priapism. However, when looking at histopathological and molecular parameters, the therapy had a positive effect on a wider range of parameters in long-term priapism. The changes in mean and maximum pressure and ICP mean/MAP ratio before and after treatment were statistically more significant in short-term priapism (Figs. 2, 3 and 4). Histopathological analysis showed a significant increase in the e-NOS expression score in the short-term ischaemic priapism, whilst there was a significant increase in the smooth muscle/collagen ratio with an increase in the e-NOS expression score in the long-term priapism. There were no significant changes in other histopathological parameters with

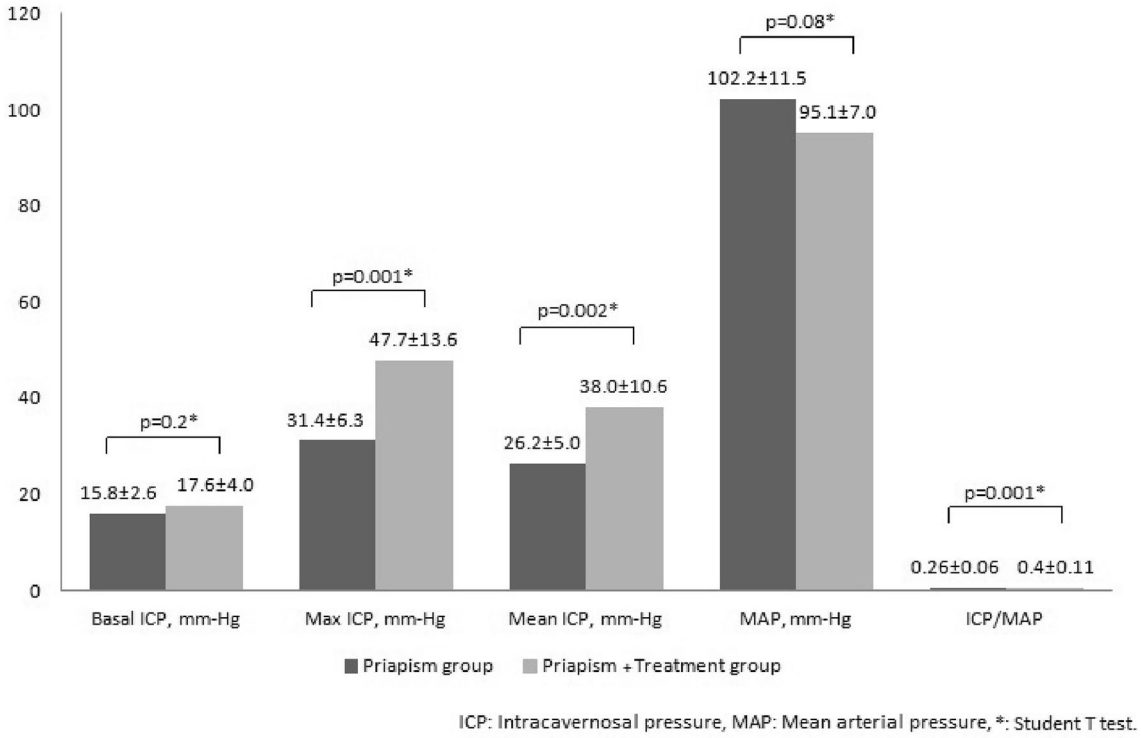

Fig. 1 Comparison of the electrophysiological parameters in the priapism and priapism + treatment groups

Table 1 Comparison of the histopathological parameters in the priapism and priapism + treatment groups

Pathological parameters	Sham group ($n=6$)	Priapism group ($n=12$)	Priapism + treatment group ($n=12$)	p
Smooth muscle/ collagen (mean ± sd)	0,67 ± 0,21	0.42 ± 0.08	0.58 ± 0.11	0.001*
Fibrosis score (median ± IQR)	0 ± 0	1 ± 1	0.5 ± 2	0.92**
TGF-beta expression score (median ± IQR)	0 ± 0	1 ± 2	1 ± 2	0.78**
Elastin expression score (median ± IQR)	2 ± 0	1.5 ± 1	1 ± 1	0.89**
e-NOS expression score (median ± IQR)	2 ± 1	1 ± 0	3 ± 1	< 0.001**
n-NOS expression score (median ± IQR)	1 ± 1	1 ± 0	2 ± 1	0.02**
i-NOS expression score (median ± IQR)	0 ± 0	1 ± 2	1 ± 1	0.23**

TGF Transforming Growth Factor, *IQR* Interquartile Range, *e-NOS* endothelial nitric oxide synthase, *n-NOS* neuronal nitric oxide synthase, *i-NOS* inducible nitric oxide synthase

* Student *t* test

**Mann–Whitney *U* test

treatment in either period (Table 3). At the molecular level, there was a significant decrease in VEGF mRNA expression and an increase in BDNF mRNA expression after treatment in the short-term priapism model, whereas all mRNA-expression parameters showed significant improvements after treatment in the long-term priapism model (Table 4).

Discussion

Prolonged ischaemic priapism left untreated or inadequately managed can lead to fibrosis of the penile corpus cavernosum, in which the smooth muscle cells are

Table 2 Comparison of the PCR findings in the in the priapism and priapism + treatment groups

PCR parameters (relative gene expressions, $2^{-\Delta\Delta ct}$)	Sham group ($n=6$)	Priapism group ($n=12$)	Priapism + treatment group ($n=12$)	p
TGF (mean ± sd)	1.04 ± 0.3	4.85 ± 3.02	2.69 ± 0.58	0.02*
VEGF (mean ± sd)	1.02 ± 0.24	3.48 ± 0.88	1.07 ± 0.8	< 0.001*
NGF (mean ± sd)	1 ± 0.13	0.61 ± 0.19	1.16 ± 0.52	0.003*
BDNF (mean ± sd)	1.01 ± 0.19	0.46 ± 0.11	1.26 ± 0.61	< 0.001*

PCR Polymerase Chain Reaction, *TGF* Transforming Growth Factor, *VEGF* Vascular Endothelial Growth Factor, *NGF* Nerve Growth Factor, *BDNF* Brain-Derived Neurotrophic Factor, *sd* standard deviation

* Student *t* test

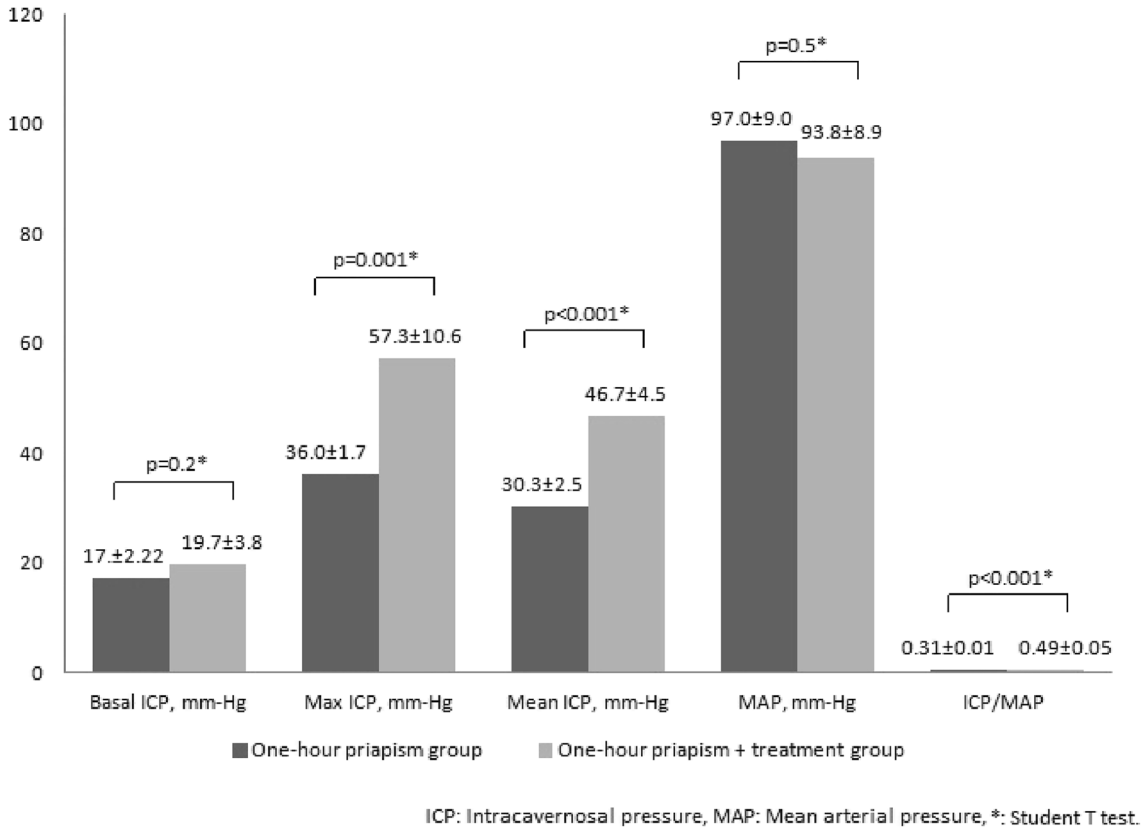

Fig. 2 Comparison of the electrophysiological parameters in 1 h priapism and 1 h priapism + treatment groups

replaced by extracellular matrix. Fibrosis ultimately leads to the loss of the ability of the cavernosal tissue to expand, resulting in erectile dysfunction [17, 18]. The initial intervention, considered a medical emergency for ischaemic priapism, is to achieve detumescence by cavernosal aspiration and prevent irreversible smooth muscle damage that can lead to erectile dysfunction [3, 19]. The aim is to reduce ischaemic damage and restore normal blood flow as soon as possible after diagnosis. However, there is currently no approved specific treatment for ischaemic priapism and secondary fibrosis caused by the ischaemic environment [19].

Theoretically, prevention of fibrosis is critical to preserving penile erectile function in patients with ischaemic priapism. A treatment that can prevent the development of penile fibrosis after ischaemia and reperfusion injury in ischaemic priapism has the potential to prevent the erectile dysfunction that will develop after priapism. Several molecules such as PDFE5I, PGE1, anti-TGF-beta, melatonin, pentoxifylline, bosentan, theophylline, zinc protoporphyrin, dippirimadol, curcumin, b-aminopropionitrile, oxytocin and pirfenidone have been used in animal models to minimise ischaemia–reperfusion injury and fibrosis in ischaemic priapism [7, 13, 20–27]. These molecules have shown promise in

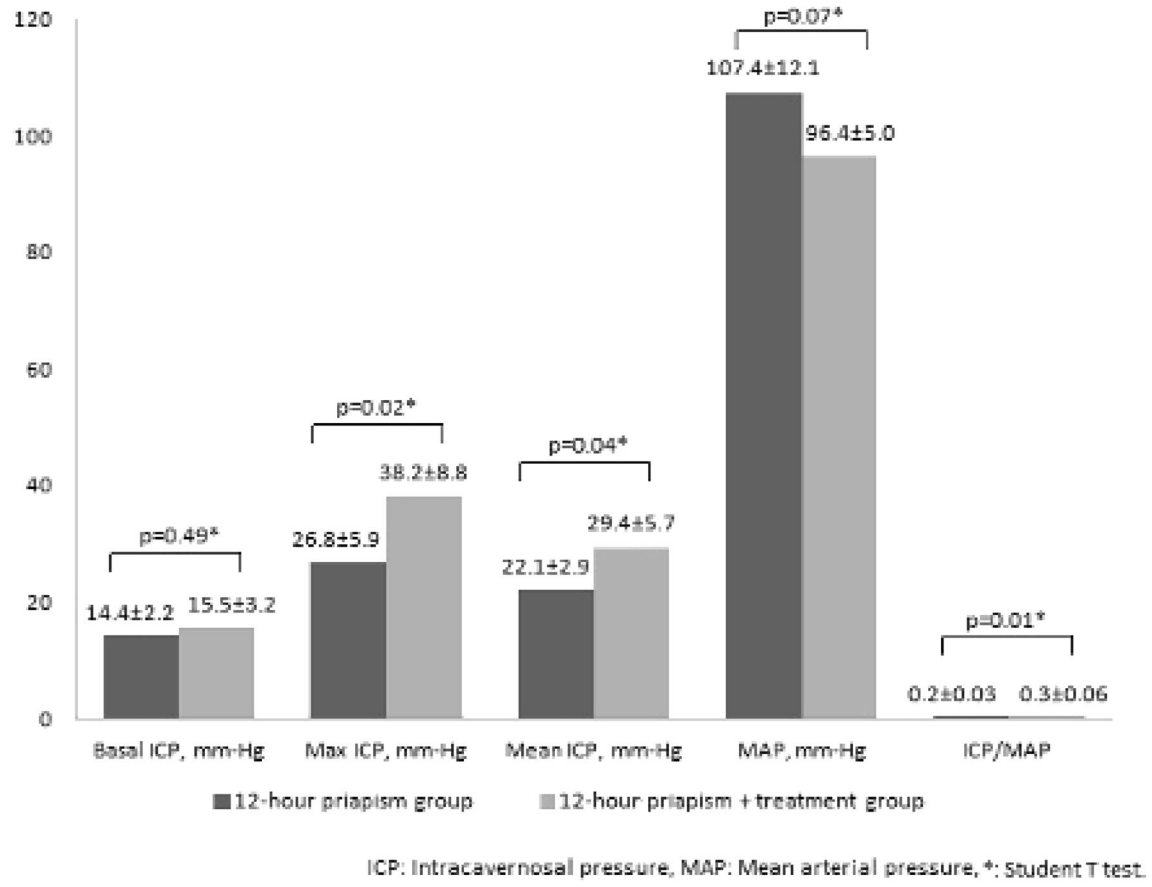

Fig. 3 Comparison of the electrophysiological parameters in 12 h priapism and 12 h priapism + treatment groups

reducing fibrosis and improving cavernosal pressure. However, none of these treatments have progressed to clinical trials.

Several experimental studies have shown that Mesenchymal stem cells (MSCs), the preferred stem-cell type for clinical use because they are easily derived from adult tissues, can reduce fibrosis in several tissues including heart, lung, liver and kidney [28–32]. MSCs exert their effects through a variety of mechanisms. Whilst differentiation and replacement of damaged cells is just one aspect, MSCs play numerous other roles in tissue repair processes. These include cell fusion, secretion of paracrine factors such as growth factors, cytokines and hormones, transfer of organelles such as mitochondria or molecules via tunnelling nanotubes, and signalling via extracellular vesicles such as exosomes and microvesicles. As a result, MSCs have demonstrated a wide range of properties, including anti-inflammatory, immunomodulatory, angiogenic, anti-apoptotic, mitotic, antifibrotic and antioxidant effects [33–35].

Given the beneficial effects of MSCs on fibrosis in other tissues, researchers have begun to explore the potential use of stem cells in the treatment of age-related fibrosis of penile tissue, Peyronie's disease and cavernosal nerve damage after prostatectomy, and diabetes [8–11]. However, the efficacy of MSC treatment for priapism has not been clearly demonstrated. To date, only one study by Siregar et al. has investigated the therapeutic role of intracavernosal MSC treatment in an ischaemic priapism model [12]. The authors showed that MSC treatment reduced the levels of TGF-beta and type 1 collagen in the cavernosal tissue of rats with the ischaemic priapism model. Siregar et al. conducted their study with a 12-h experimental priapism period and aimed to evaluate the potential antifibrotic effect of the treatment [12]. However, they did not describe the intracavernosal pressure changes and histopathological effects during the priapism model and after treatment.

In the present study, we aimed to comprehensively determine the therapeutic efficacy of intracavernosal MSC therapy by investigating its physiological, histological and molecular effects in a short-term and long-term ischaemic priapism model. Studies using this method have shown that the most common priapism model lasts 1 h, whilst the longest lasting model is 24 h [13, 27]. In our study, we used 1- and 12-h models to assess the effectiveness of treatment in short- and long-term priapism, providing a more detailed evaluation. In addition, we used a

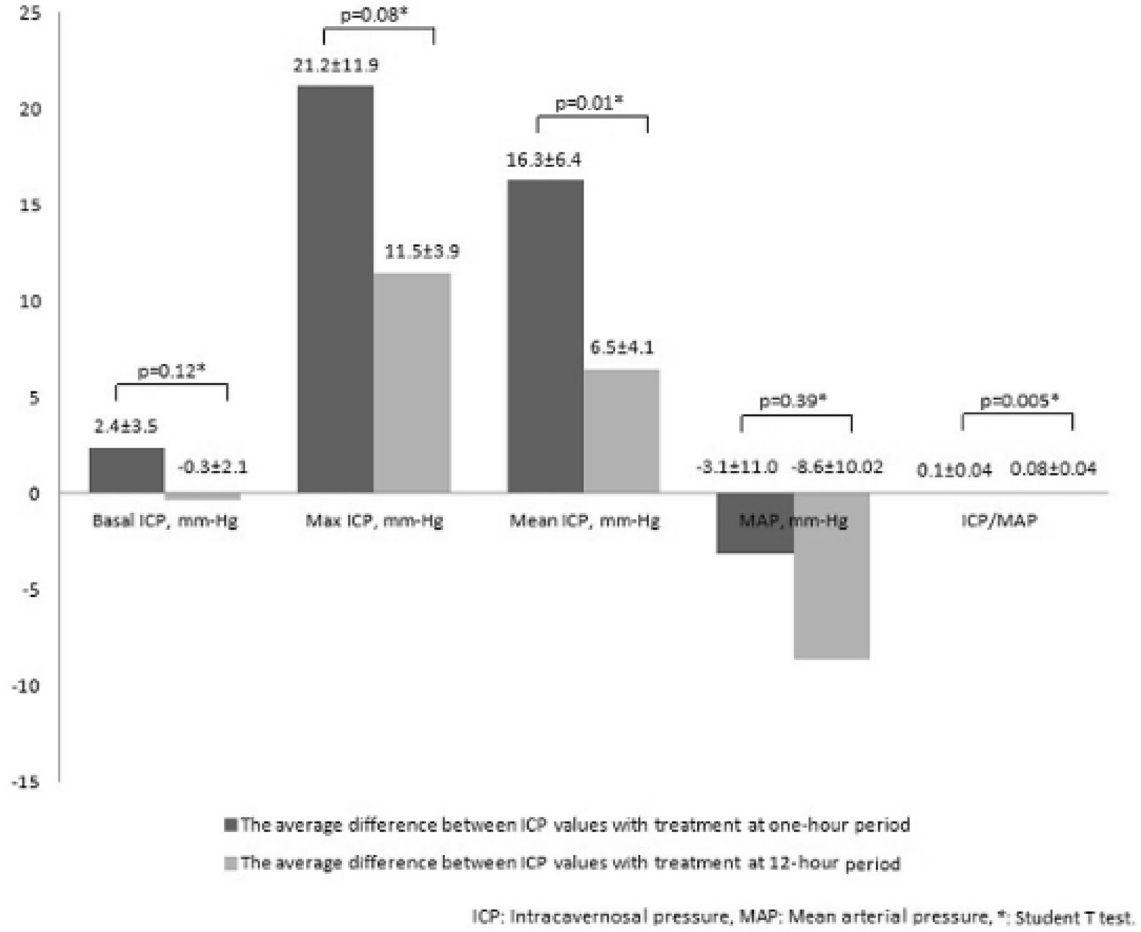

Fig. 4 Statistical analysis of the differences in pressure values_control and treatment groups (1 and 12 h subgroups)

sham group in which intracavernosal saline was applied to verify the successful priapism model. In addition, we objectively evaluated the erectile function of the rats by measuring ICPs after electrical stimulation of the cavernosal nerve as previously described by Quinlan et al. [16]. The results showed a significantly lower mean ICP/MAP ratio in the priapism group compared to the sham group. Histopathologically, we found increased fibrosis and TGF-beta expression scores with decreased elastin and e-NOS expression scores in the priapism group. Our PCR analysis results also showed significant increases and decreases in TGF-beta and VEGF mRNA expression and NGF and BDNF mRNA expression in the priapism model. Therefore, our results suggest that we have successfully created the model. Intracavernosal MSC treatment increased maximum and mean ICPs and the ICP-mean/MAP ratio in both the short-term and long-term priapism groups. Subgroup analysis revealed greater increases in mean ICP and ICP mean/MAP in the 1-h priapism model, suggesting that the treatment was more effective on penile erection physiology in short-term priapism. In our histopathological examination, the cavernosal smooth muscle to collagen ratio showed a significant decrease in rats with priapism and increased after treatment, especially in rats with long-term priapism (Fig. 5). These findings suggest that increasing the smooth muscle to collagen ratio by intracavernosal MCS treatment improved cavernosal tissue compliance and increased cavernosal pressure. However, increased fibrosis and TGF-beta expression scores and decreased elastin expression scores in the short-term and long-term priapism groups were not normalised by MCS application. These results suggest that prolonged priapism caused more damage, as expected, and therefore the therapeutic effect of the treatment was a sum of limitation. On the other hand, the expression of e-NOS, but not n-NOS and i-NOS, in the cavernosal tissue in both short-term and long-term priapism models showed a significant increase with MCS treatment (Fig. 6). As e-NOS plays an important role in erection physiology and its expression is known to be associated with erectile dysfunction, it is a key molecule in penile physiology [36, 37]. In this context, our results suggest that improved erectile function via additional

Table 3 Comparison of the histopathological parameters in the 1 and 12-h priapism and treatment groups

Pathological parameters	1-h priapism group ($n=6$)	1-h priapism + treatment group ($n=6$)	p	12-h priapism group ($n=6$)	12-h priapism + treatment group ($n=6$)	p
Smooth muscle/collagen (mean ± sd)	0.44 ± 0.07	0.57 ± 0.12	0.05*	0.41 ± 0.09	0.58 ± 0.11	0.01*
Fibrosis score (median ± IQR)	0 ± 0	0 ± 0	0.3**	1 ± 0	1 ± 1	0.4**
TGF-beta expression score (median ± IQR)	0.5 ± 1	0.5 ± 1	0.9**	1.5 ± 1	1 ± 1	0.78**
Elastin expression score (median ± IQR)	2 ± 0	2 ± 1	0.13**	1 ± 1	1 ± 1	0.33**
e-NOS expression score (median ± IQR)	1 ± 1	3 ± 1	0.004**	1 ± 0	3 ± 1	0.001**
n-NOS expression score (median ± IQR)	1 ± 2	2 ± 1	0.16**	1 ± 0	1 ± 1	0.05**
i-NOS expression score (median ± IQR)	0.5 ± 1	1.5 ± 1	0.07**	1 ± 1	1 ± 1	0.52**

TGF Transforming Growth Factor, *IQR* Interquartile Range, *e-NOS* endothelial nitric oxide synthase, *n-NOS* neuronal nitric oxide synthase, *i-NOS* inducible nitric oxide synthase

* Student *t* test

** Mann–Whitney *U* test

Table 4 Comparison of the PCR findings parameters in the 1 and 12-h priapism and treatment groups

PCR Parameters (relative gene expressions, $2^{-\Delta\Delta ct}$)	1-h priapism group ($n=6$)	1-h priapism + treatment group ($n=6$)	p	12-h priapism group ($n=6$)	12-h priapism + treatment group ($n=6$)	p
TGF (mean ± sd)	2.09 ± 0.53	2.24 ± 0.48	0.6*	7.61 ± 1.2	3.12 ± 0.2	< 0.001*
VEGF (mean ± sd)	2.93 ± 0.34	0.41 ± 0.43	< 0.001*	4.03 ± 0.94	1.73 ± 0.45	< 0.001*
NGF (mean ± sd)	0.61 ± 0.19	0.7 ± 0.14	0.3*	0.62 ± 0.21	1.61 ± 0.3	< 0.001*
BDNF (mean ± sd)	0.48 ± 0.13	0.71 ± 0.18	0.03*	0.45 ± 0.1	1.82 ± 0.25	< 0.001*

PCR Polymerase Chain Reaction, *TGF* Transforming Growth Factor, *VEGF* Vascular Endothelial Growth Factor, *NGF* Nerve Growth Factor, *BDNF* Brain-Derived Neurotrophic Factor, sd standard deviation

*Student *t* test

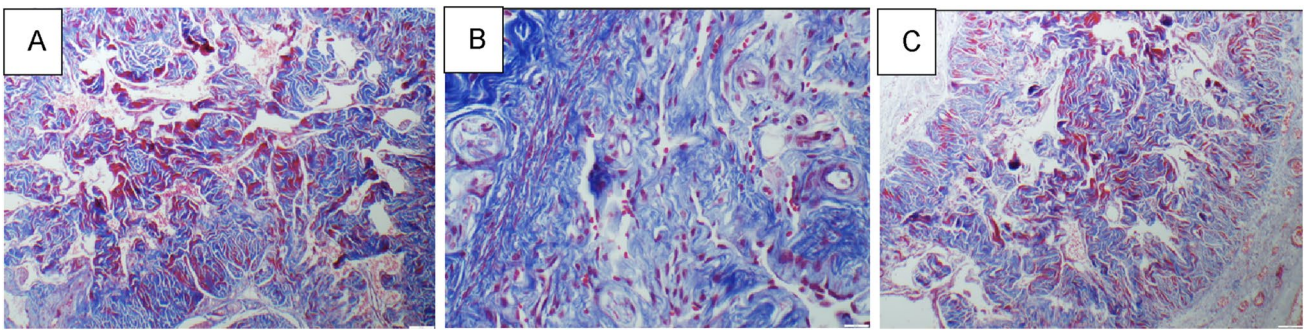

Fig. 5 Increase in smooth muscle/collagen ratio with MSC treatment (**A** sham group, **B** priapism group, **C** MSC treatment group) (×200)

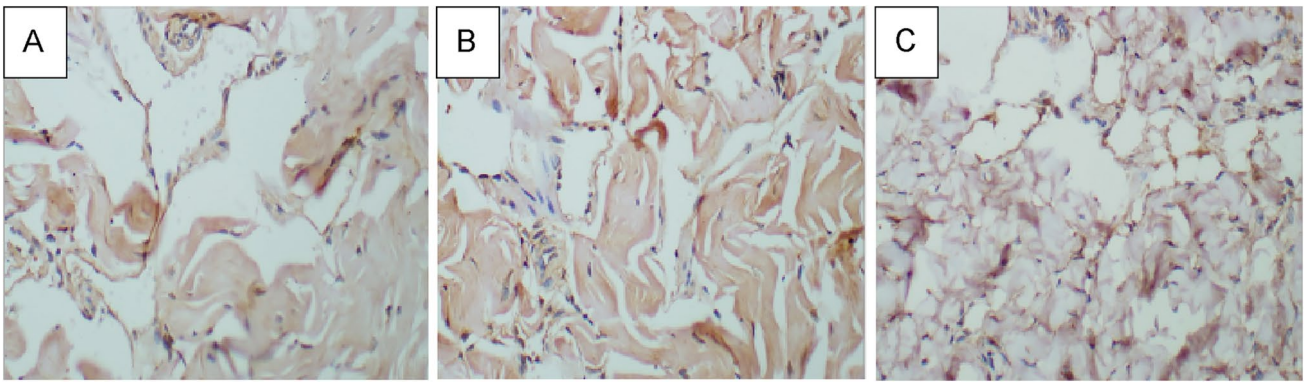

Fig. 6 Increase in e-NOS expression scores with MSC treatment (**A** sham group, **B** priapism group, **C** MSC treatment group) (×400)

enhanced e-NOS expression with intracavernosal MCS treatment in the ischaemic priapism model.

TGF-beta is a key cytokine in the development of fibrosis and its release is increased under hypoxic conditions and previous studies have shown an association between TGF-beta and penile fibrosis [13, 38]. We did not observe a significant decrease in tissue levels of TGF-beta with MSC treatment in either short-term or long-term ischaemic priapism. On the other hand, our PCR results showed a significant increase in cavernosal tissue TGF-beta mRNA expression with treatment in the long-term priapism models. In our opinion, these results suggest that the antifibrotic process had already started at the molecular level and that changes at the tissue level would take more time. With presented study, MSC may be useful not only in protecting against the complications of priapism but also in protecting against many other conditions that may cause penile fibrosis. Penile fibrosis and erectile dysfunction are the major complications of radical prostatectomy, which is frequently used in the treatment of prostate cancer, and of 5-alpha-reductase inhibitors, which are frequently used in the treatment of benign prostatic hyperplasia. Kilic et al. demostrated the detrimental effects of 5-alpha-reductase inhibitors on penile collagen deposition and erectile function in a rat model [39]. In addition Oral et al. reported that adipose-derived stem cells improve cavernosal functions after radical prostatectomy in a rat model [40]. The presented research offers a promising alternative regenerative treatment for penile rehabilitation, with the potential to reduce fibrosis and improve cavernosal function.

In addition, the expression level of intracavernosal VEGF, a signalling protein secreted by many tissues in hypoxia and involved in inflammation, vasculogenesis and angiogenesis, was increased by the creation of a hypoxic environment in our ischaemic priapism model [41]. The increase was greater in the long-term period and showed a significant decrease with MCS treatment in both periods. In conclusion, intracavernosal MSC application provided anti-fibrotic and anti-inflammatory properties in hypoxic tissue by suppressing TGF-beta and VEGF expression, especially in long-term ischaemic priapism. NGF and BDNF are members of the neurotrophin family of growth factors and both molecules are responsible for cellular survival and differentiation of neurons in the central and peripheral nervous system [42]. They also play an important role in erectile physiology [43]. It is known that NGF and BDNF levels are reduced in cavernosal nerve injury and diabetes-related erectile dysfunction [44, 45]. Similarly, in the present study, we found significantly reduced levels of these molecules in the damaged cavernosal tissue of rats with ischaemic priapism. However, it is important to note that intracavernosal MSC treatment significantly increased the levels of these neuronal molecules, especially in the long-term priapism model. This suggests that MSC treatment contributed to the improvement of the neuronal component of penile erectile function in rats with ischaemic priapism.

A limitation of this study is the small number of rats in the sham and priapism subgroups ($n=6$). Although a power analysis was conducted and the number of rats was determined to be sufficient to achieve statistical significance, the small sample size in these groups may limit the generalizability of the findings. Given the high variability typically observed in such studies, it would have been beneficial to use a larger number of animals to better account for potential variability in the data. However, this study adhered to ethical standards regarding animal use, balancing the need for scientific rigour with the ethical obligation to minimise the number of animals used.

In conclusion, MSC treatment improved cavernosal physiology and had positive effects at histopathological and molecular levels in the ischaemic priapism model. It also contributed to the neuronal component of erectile function. Further research, including randomised controlled human trials, is needed before intracavernosal MSC therapy can be implemented in clinical practise.

Author contribution Study conception and design: AK, EK, AÇ, AYM, EK. Material preparation: YB, MZT, SY. Data collection: HHT, EK, MZT. Analyses: ZBG, ŞÖ. The first draught of the manuscript was written by EK, AÇ, MD, AK and EK. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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